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Clinical Correlation Between WISP2 and β-Catenin in Gastric Cancer

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Abstract. *Background: Evidence indicates that wingless-type MMTV integration site family, member 1 (WNT1)-inducible signaling pathway protein 2 (WISP2) may play an important role in the development of gastric cancer (GC) by regulating the WNT/β-catenin signaling pathway. In the present study, we investigated whether there is correlation between WISP2 and β-catenin proteins, and their association with clinicopathological features in GC. Materials and Methods: Immunohistochemical staining was carried out on 119 paraffin-embedded gastric cancer tissues and 99 adjacent normal gastric tissues collected from patients with GC at the Beijing Cancer Hospital. Data were analyzed by Spearman rank correlation and Chi-square tests. Results: Both WISP2 and β-catenin were more highly expressed in GC tissues compared to adjacent normal tissues. Moreover, Spearman rank correlation analysis showed positive correlation between WISP2 and β-catenin (R=0.2254, p=0.0137). Additionally, their co-expression was seen in a higher proportion of patients with GC at early stage or without metastasis. Conclusion: These findings suggest that the expression of WISP2 and β-catenin might be a favorable biomarker for prediction and prognosis in the early stage of GC.*

Gastric cancer (GC) remains the fourth most common malignant tumor and the second frequent cause of cancer death worldwide (1). The majority of patients do not typically present symptoms until the later stages of GC, consequently when tumor diagnosis is confirmed, the disease has usually progressed to the advanced stage with metastasis to distant organs. Although there has been much improvement in management of GC, most patients with advanced disease still face poor prognosis and a high mortality rate (2). Thus, improving early detection has become essential for effective treatment and therefore useful diagnostic biomarkers for GC are urgently needed.

Wingless-type MMTV integration site family, member 1 (WNT1)-inducible signaling pathway protein-2 (WISP2) is one protein belonging to the CCN (cysteine-rich glycosylated signaling protein) family. WISP2 exhibits various expression profiles in different human cancer types, and are even in tumor stage-specific and subtype-specific. A lower level of WISP2 expression was found in pancreatic and colorectal carcinoma compared to adjacent normal tissues (3, 4). In contrast, WISP2 is overexpressed in hepatocarcinoma, skin cancer and pituitary tumors (5-7). Intriguingly, Banerjee *et al.* found that WISP2 expression in breast cancer was biphasic and higher WISP2 expression was detected in less aggressive human breast cancer cells (8). Due to its special expression profile in breast cancer, WISP2 has been highlighted as a useful indicator of breast cancer progression (9). GC exhibits a similar WISP2 expression prolife in that a significant decrease was seen in poorly differentiated tumors compared with well- and moderately differentiated tumors (10).

WISP2 plays multiple pathophysiological roles in tumor progression, including cell proliferation, motility, invasiveness, adhesion and epithelial–mesenchymal transition (EMT) (11). Accumulating data suggest that there may be a reciprocal inhibition between WISP and EMT. In GC, WISP2 repressed cell motility, growth and invasion through down-regulations of EMT *via* c-Jun *N*-terminal kinase, extracellular signal-regulated kinase and phosphoinositide phospholipase C (PLC-γ) signaling pathways (10). Furthermore, Capietto *et al.* reported that deficiency of PLC-γ is likely to cause a reduction in β-catenin expression, which has been verified in myeloid-

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derived suppressor cells in patients with tumors (12). It seems that there is a link between WISP2 and β-catenin.

β-Catenin is the core component of the canonical WNT signaling pathway, which plays key functions in the regulation of cell growth, proliferation, morphology, motility and organ development (13). EMT is another phenotype caused by β-catenin activation and β-catenin triggers cancer cells migration *via* EMT marker alterations, such as E-cadherin, N-cadherin, matrix metallopeptidase 2 (MMP2) and MMP9 (14). However, to date, the relationship between WISP2 and β-catenin in the development of GC is poorly understood. In this study, we detected the expression of WISP2 and β-catenin in GC tissues, and also investigated the clinical associations between the two molecules.

Materials and Methods

Patient and gastric tissue specimens. A total of 119 paraffin-embedded GC tissues and 99 adjacent normal gastric tissues were collected at Peking University Beijing Cancer Hospital from 2003 to 2007. The tissue collection from 218 patients was approved by the Ethics Committee of Beijing Cancer Hospital. All patients signed informed consent forms for use of their tissue and data.

Immunohistochemistry. All samples were analyzed by immunohistochemistry. Tissue sections of 4-μm thickness were cut from formalin-fixed paraffin-embedded specimens were deparaffinized with xylene and rehydrated with graded ethanol solutions. After antigen retrieval was conducted by autoclaving in 0.01 M citrate buffer (pH 6.0) for 3 minutes and cooling for 5 minutes, sections were covered in 3% hydrogen peroxide in methanol for 10 minutes to block endogenous peroxidase at room temperature. The tissue slides were then incubated with serum-blocking solution for 90 min at 37˚C, and polyclonal antibody to WISP2 (1:200) and β-catenin monoclonal antibody (1:500) (Abcam, Cambridge, UK) overnight at 4˚C, followed by secondary antibody for 30 min at 37˚C. Slides were visualized using diaminobenzidine, counterstained with hematoxylin, dehydrated in ethanol and cleared with xylene. Samples were considered positive when 10% or more of the cells had cytoplasmic staining for WISP2 or β-catenin. The expression of WISP2 or β-catenin was assessed independently by two experienced pathologists who were blind to the patients' clinical outcomes.

Statistical analysis. All data were analyzed by SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Spearman rank correlation was used to evaluate the correlation between WISP2 and β-catenin expression. Chi-squared test was carried out to clarify the relationship between WISP2 and β-catenin expression and clinicopathological features in GC. Two-tailed *p*-values of less than 0.05 were considered statistically significant.

Results

WISP2 levels are positively correlated with β-catenin in GC. A total of 119 GC tissues and 99 adjacent normal tissues in total were assessed by immunohistochemistry in order to explore the correlation between WISP2 and β-catenin. Figure 1 shows representative images of WISP2 and β-catenin staining in GC, respectively. In GC samples, WISP2 showed higher expression compared to adjacent normal tissues (p=0.0001), but total β-catenin expression did not differ significantly (p=0.1549) (Table I).

According to our rating criteria of the immunostaining (+ 10-40%; ++ 41-60%; +++ >60%), 21(17.6%) GC samples had simultaneous negative expression of both WISP2 and β-catenin, and 46 (38.8%) had positive expression for both WISP2 and β-catenin. In addition, 47 (39.4%) of the WISP2-negative samples were positive for β-catenin expression, however, only five (4.2%) WISP2-positive samples were negative for β-catenin expression (Table II).

Using Spearman rank correlation analysis, we found that there was positive correlation between WISP2 and β-catenin expression in GC (R=0.2254, p=0.0137) (Table II). Furthermore, our analysis showed that expression of these two molecules was more strongly positively correlation in the early stage(I and II) than the advanced stage (III and IV) of GC (R=0.3275 and R=0.0975, and p=0.0078 and p=0.4911, respectively) (Table III). In addition, there was no statistical significance of correlation between WISP2 and β-catenin expression in samples of adjacent normal tissue (R=0.1986, p=0.578) (data not shown).

The effect of co-expression of WISP2 and β-catenin on clinicopathological features of patients with GC. In order to investigate the role of WISP2 and β-catenin expression in gastric carcinogenesis, we examined whether the co-expression of WISP2 and β-catenin correlated with clinicopathological characteristics in patients with GC. As shown in Table IV, GC tissues with less tumor infiltration (T1-T2) exhibited significantly higher rate of co-expression of WISP2 and β-catenin compared to those with more tumor infiltration (T3-T4) (61.1% *vs*. 34.6%, p=0.0337). Likewise, the majority of GC cases without distinct metastasis showed much more co-expression of WISP2 and β-catenin than those with distinct metastasis (44.2% *vs*. 16.7%, p=0.0133). Additionally, in the group of patients with GC who survived longer than 5 years, co- expression of WISP2 and β-catenin tended to be slightly higher without significance (51.4% *vs*. 33.3%, p=0.0647). However, the co-expression did not reach statistical significance in regard to lymph node metastasis, not was it associated with age or gender.

Discussion

WISP2 is a member of the CCN family of cysteine-rich, glycosylated signaling proteins, which mediate diverse developmental processes (14). A previous study demonstrated elevated expression of WISP2 in GC compared to normal tissues, which was associated with good prognosis in patients with GC (10). It has also been reported that WISP2 inhibited

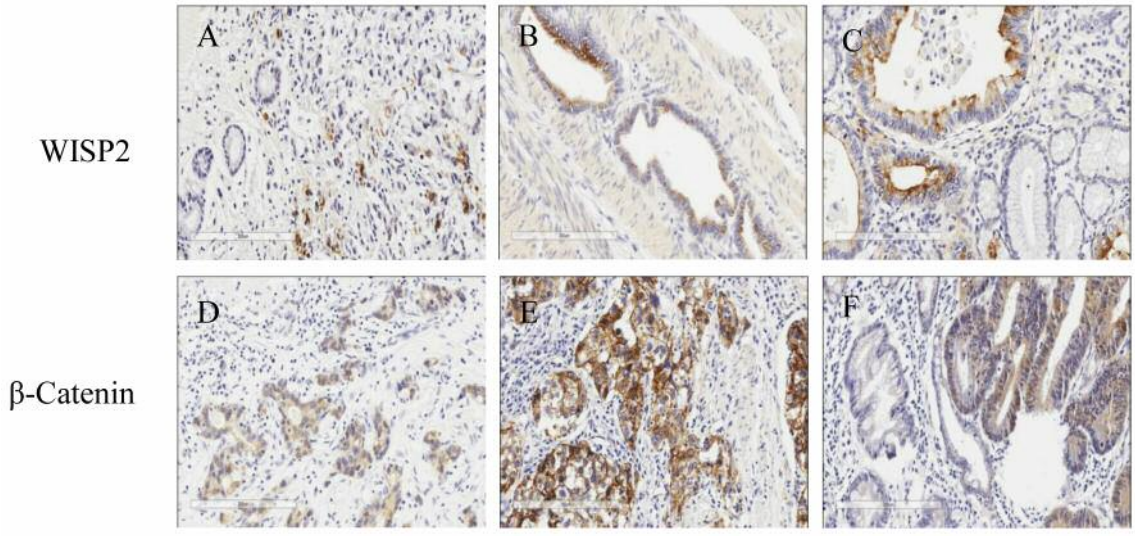

Figure 1. *Representative images of staining for wingless-type MMTV integration site family, member 1-inducible signaling pathway protein 2 (WISP2) (A-C) and β-catenin (D-F) in gastric cancer. WISP2 staining in non-gastric gland section (A), and gastric gland section (B-C). Weak (D), strong (E) and intermediate (F) staining of β-catenin in gastric gland sections. Scale bar=300 μm.*

Table I. *Immunohistochemical staining of WNT1-inducible signaling pathway protein (WISP2) and β-catenin expression in gastric cancer versus normal tissues.*

Tissue	WISP2		*p*-Value	β-Catenin		*p*-Value
	Negative	Positive		Negative	Positive	
Gastric cancer	70 (59%)	49 (41%)	0.0001	26	93	0.1549
Normal tissue	82 (83%)	17		30	69	

PLC-γ signaling pathway, which in turn reduces β-catenin expression to affect EMT (12). However, the relationship between WISP2 and β-catenin is still not clear.

As a key molecule in the WNT signaling pathway, β-catenin protein is found to be distributed in the membrane, cytoplasm and nucleus (15). β-Catenin has a dual role in cancer, depending on its intracellular localization. Membranous β-catenin is responsible for cell-to-cell adhesion, exerting a restrictive effect on tumor growth by linking with E-cadherin and actin cytoskeleton. Cytoplasmic and nuclear β-catenin are mainly involved in regulation of the WNT signaling pathway (16). The binding of WNT with its receptor (*e.g.* Frizzled) can inhibit destruction of the β-catenin complex, and as a consequence, free cytoplasmic β-catenin accumulates and is translocated into the nucleus, where β-catenin initiates transcription of target genes associated with tumor development and metastasis, such as regulation of EMT (17). Dysregulation of β-catenin has been seen in the development of multiple tumors, including in GC. The multiple cellular localization of β-catenin makes the relationship between β-catenin and clinicopathological parameters controversial in GC according to the literature. For instance, GC tumors lacking membranous expression of β-catenin have greater extent of lymph node metastasis, poor differentiation and advanced T-stage (18). On the other hand, abnormalities of β-catenin in cytoplasm or nuclear tend to be associated with poor survival (19).

In this study, we show that total β-catenin expression appears not to differ in GC and adjacent normal tissues. Spearman correlation analysis revealed positive correlation of WISP2 with β-catenin levels in GC tissues, especially in the early stage. Furthermore, the co-expression of WISP2 and β-catenin was at a much higher rate in GC tissues at the early stage or without metastasis. A previous study reported similar results that abnormal co-expression of E-cadherin and β-catenin frequently occurs in early gastric carcinogenesis and might play an important role in the mode of metastasis of early GC (20).

Table II. *Positive correlation between WNT1-inducible signaling pathway protein 2 (WISP2) and β-catenin expression in gastric cancer.*

WISP2	β-Catenin		Total	R	*p*-Value
	Negative	Positive			
Negative	21 (17.6%)	47 (39.4%)	68	0.2254	0.0137
Positive	5 (4.2%)	46 (38.8%)	51		
Total	26	93	119		

Table III. *The correlation between WNT1-inducible signaling pathway protein 2 (WISP2) and β-catenin expression in different stage of gastric cancer.*

Stage	R	*p*-Value
Early stage (I+II)	0.3275	0.0078
Advanced stage (III+IV)	0.0957	0.4911

Table IV. *Association between the co-expression of WNT1-inducible signaling pathway protein 2 (WISP2) and β-catenin with clinicopathological parameters in gastric cancer.*

Group	Co-expression	Neither	Chi-squared	*p*-Value
Gender				
Male	30 (40.5%)	44 (59.6%)	0.293	0.5415
Female	16 (35.6%)	29 (64.4%)		
Age				
≤60 Years	27 (41.5%)	38 (58.5%)	0.502	0.4786
>60 Years	19 (35.2%)	35 (64.8%)		
Depth of invasion				
T1+T2	11 (61.1%)	7 (38.9%)	4.510	0.0337
T3+T4	35 (34.6%)	66 (65.4%)		
Lymph node metastasis				
No	13 (39.3%)	20 (60.7%)	0.010	0.9182
Yes	33 (38.3%)	53 (61.7%)		
Distant metastasis				
M0	42 (44.2%)	53 (55.8%)	6.130	0.0133
M1	4 (16.7%)	20 (83.3%)		
Survival rate				
>5 Years	18 (51.4%)	17 (48.6%)	3.411	0.0647
≥5 Years	28 (33.3%)	56 (66.7%)		

Taken together, our data suggest that co-expression of WISP2 and β-catenin may be used as favorable biomarkers for the prediction and prognosis particularly in the early stage of GC, and might be a hallmark of early metastasis of GC. However, we would also like to state several limitations involved in our study which may render potential bias. Firstly, the patient samples were collected from a single center, and the sample size may not be adequate when they are classified into different GC groups. Secondly, we only analyzed the total expression levels of β-catenin in GC tissues, ignoring its intracellular localization, due to technical restriction of immunostaining we used. To the best of our knowledge, this is the first report describing the correlation between WISP2 and β-catenin proteins in GC. It might be worth investigating the mechanism underlying the co-expression of WISP2 and β-catenin in GC, which may be valuable for earlier or more accurate diagnosis of this malignancy.

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